PATENT COOPERATION TREATY

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

From the INTERNATIONAL BUREAU

To:

GIMMI, Edward, R.
SmithKline Beecham Corporation
Corporate Intellectual Property,
UW2220
709 Swedeland Road
P.O. Box 1539
King of Prussia, PA 19406-0939
ETATS-UNIS D'AMERIQUE

Date of mailing (day/month/year)
16 August 2001 (16.08.01)

Applicant's or agent's file reference
GM50057

IMPORTANT NOTIFICATION

International application No.
PCT/US00/12133

International filing date (day/month/year)
04 May 2000 (04.05.00)

1. The following indications appeared on record concerning:

[X] the applicant [X] the inventor [ ] the agent [ ] the common representative

Name and Address	State of Nationality	State of Residence
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[X] the person [X] the name [X] the address [X] the nationality [X] the residence

Name and Address	State of Nationality	State of Residence
NURSE, Kelvin, C. 1366 Knox Drive Yardley, PA 19067 United States of America	US	US
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	

3. Further observations, if necessary:
The above-mentioned person has been added to the records as applicant/inventor for US only.

4. A copy of this notification has been sent to:

[X] the receiving Office
[ ] the International Searching Authority
[X] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[X] the elected Offices concerned
[ ] other:

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer A. Karkachi
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

10/019364

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference GM50057	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/US00/12133	International filing date (day/month/year) 04 MAY 2000	Priority date (day/month/year) 20 MAY 1999
International Patent Classification (IPC) or national classification and IPC IPC(7): C07H 21/02, 21/04; A01N 61/00 and US Cl.: 536/23.1; 514/1		
Applicant SMITHKLINE BEECHAM CORPORATION		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets.

   ☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority. (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 0 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of report with regard to novelty, inventive step or industrial applicability
   - IV ☒ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☒ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
08 NOVEMBER 2000	05 SEPTEMBER 2001
Name and mailing address of the IPEA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer JOSEPH T. WOITACH Telephone No. (703) 308-0196

Form PCT/IPEA/409 (cover sheet) (July 1998)*

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US00/12133

I. Basis of the report

1. With regard to the **elements** of the international application:*

☐ the international application as originally filed

☒ the description:
pages 1-32 , as originally filed
pages NONE , filed with the demand
pages NONE , filed with the letter of _____

☒ the claims:
pages 33-37 , as originally filed
pages NONE , as amended (together with any statement) under Article 19
pages NONE , filed with the demand
pages NONE , filed with the letter of _____

☒ the drawings:
pages 1-23 , as originally filed
pages NONE , filed with the demand
pages NONE , filed with the letter of _____

☒ the sequence listing part of the description:
pages NONE , as originally filed
pages NONE , filed with the demand
pages NONE , filed with the letter of _____

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language _____ which is:

☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

☐ the language of publication of the international application (under Rule 48.3(b)).

☐ the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

☐ contained in the international application in printed form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ The statement that the information recorded in computer readable form is identical to the writen sequence listing has been furnished.

4. ☒ The amendments have resulted in the cancellation of:

☒ the description, pages NONE

☒ the claims, Nos. NONE

☒ the drawings, sheets/~~fig~~ NONE

5. ☐ This report has been drawn as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

** Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

***Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.*

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

- [ ] restricted the claims.
- [ ] paid additional fees.
- [ ] paid additional fees under protest.
- [ ] neither restricted nor paid additional fees.

2. [X] This Authority found that the requirement of unity of invention is not complied with and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

- [ ] complied with.
- [X] not complied with for the following reasons:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1.

Group 1, claim(s)1-7, drawn to an isolated polynucleotide set forth in SEQ ID NO: 1, said polynucleotide in a vector, and said vector in a host cell.
Group 2, claim(s) 8, drawn to a method of treating an individual in need of a ribosomal inhibitor.
Group 3, claim(s) 9-10, 13 and 14, drawn to a method of identifying compounds which interact and inhibit or activate the polynucleotide set forth in SEQ ID NO: 1.
Group 4, claim(s)11, 12 and 15, drawn to a method of treating an individual infected by a bacteria.

The inventions listed as Groups 1-4 do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: A polynucleotide which is 70% homologous to the polynucleotide set forth in SEQ ID NO: 1 has previously been disclosed (23S rRNA-Accession # V00331). Further, the existence of a polynucleotide would not anticipate that an inhibitor or activator of activity exist, nor that if such a compound existed that it could be used in treatment of an individual.

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

- [X] all parts.
- [ ] the parts relating to claims Nos. _.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. statement

Novelty (N)	Claims	8, 11, 12	YES
	Claims	1-7, 9-10, 13-15	NO
Inventive Step (IS)	Claims	8, 11, 12	YES
	Claims	1-7, 9-10, 13-15	NO
Industrial Applicability (IA)	Claims	1-15	YES
	Claims	NONE	NO

2. citations and explanations (Rule 70.7)

Claims 1-7 lack novelty under PCT Article 33(2) as being anticipated by the polynucleotide sequence disclosed in Accession number V00331 (Genbank entry). Claim 1-7 encompass a polynucleotide which is 70% homologous to SEQ ID NO: 1. The E. coli gene rrnB codes for the 23S RNA. This nucleotide had been isolated and sequenced.

Claims 9-10, 13-15 lack novelty under PCT Article 33(2) as being anticipated by Hogenauer et al. and Dornhelm et al. in view of V00331. Hogenauer et al. and Dornhelm et al. disclose methods for detecting antibiotic compounds. Specifically, methods detailing how to determine stoichiometry, location and affinity for various analogs are disclosed for various ribosomal subunits of E. coli. In view of each of these, it would be obvious to determine if these specific compounds interact with the 23S RNA, and in addition, to use the general methodology to determine if new analogs/compounds would specifically inhibit the 23S subunit.

Claims 1-15 have industrial applicability as defined by PCT Article 33(4) because new antibacterial compounds are recognized to be useful in treating bacterial infections, in particular bacteria which have become resistant to known antibiotics.

Claims 8, 11 and 12 meet the criteria set out in PCT Article 33(2)-(4), because the prior art does not teach or fairly suggest the use of the compounds defined by their ability bind the polynucleotide set forth in SEQ ID NO: 1 for use in treatment of an individual, however the application fails to clearly define these compounds as noted in the objection under PCT Rule 66.2(a)(iii).

(Continued on Supplemental Sheet.)

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

The description is objected to as containing the following defect(s) under PCT Rule 66.2(a)(iii) in the form or contents thereof: The specification provides guidance to discover new compounds which bind to the polynucleotide sequence set forth in SEQ ID NO: 1, and to determine if they are agonists or antagonists of activity, however there is no clear guidance demonstrating that use of these compounds whose function has been determined in vitro, could be used in vivo for treatment of a patient. There are no structural or functional limitations given to the compound which would indicate that it could be administered for treatment.

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

Claims 10, 13 and 15 are objected to under PCT Rule 66.2(a)(v) as lacking clarity under PCT Article 6 because the claim indefinite for the following reason(s): The compound and methods of identifying said compound or using said compound to treat an individual are unclear because functional limitations are recited that in claim 10 describing the compound. Since a specific compound is not recited and it can be either an antagonist or an agonist, it is unclear that if a compound meets one of the functional limitations if it would necessarily reuslt in a compound which could be used for treatment. Further, a polynucleotide sequence which is 70% homologous may have no functional relationship to the endogenous sequences and therefore the compouds which are functional identified with these sequences may not interact with the native sequence at all.

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: Boxes I - VIII

Sheet 10

V. 2. REASONED STATEMENTS - CITATIONS AND EXPLANATIONS (Continued):

---------- NEW CITATIONS ----------
NONE

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(71) Applicants *(for all designated States except US)*: **SMITHKLINE BEECHAM CORPORATION** [US/US]; One Franklin Plaza, Philadelphia, PA 19103 (US). **SMITHKLINE BEECHAM PLC** [GB/GB]; New Horizons Court, Great West Road, Brentford, Middlesex TW8 9EP (GB).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **HEGG, Lisa, Anne** [US/US]; 554 Tanglewood Lane, Devon, PA 19333 (US). **STERNER, Therese, Anne** [US/US]; 340 Sugartown Road, Devon, PA 19333 (US).

(74) Agents: **GIMMI, Edward, R.** et al.; SmithKline Beecham Corporation, Corporate Intellectual Property, UW2220, 709 Swedeland Road, P.O. Box 1539, King of Prussia, PA 19406-0939 (US).

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 00/71560 A1

(54) Title: METHODS OF MODULATING ACTIVITY OF PROKARYOTIC RIBOSOMES

(57) Abstract: This invention relates to newly identified polynucleotides and interactions of these polynucleotides with polypeptides, and their production and uses, as well as their variants, agonists and antagonists, and their uses. In particular, in these and in other regards, the invention relates to polynucleotides used in identifying compounds that modulate the activity of prokaryotic ribosomes.

What is claimed is:

1. An isolated polynucleotide comprising a polynucleotide sequence selected from the group consisting of:

(a) a polynucleotide having at least a 70% identity to a polynucleotide comprising the nucleotide sequence of SEQ ID NO:1; or

(b) a polynucleotide which is complementary to the polynucleotide of (a).

2. The polynucleotide of Claim 1 wherein the polynucleotide is DNA.

3. The polynucleotide of Claim 1 wherein the polynucleotide is RNA.

4. The polynucleotide of Claim 2 comprising the nucleic acid sequence set forth in SEQ ID NO:1.

5. A vector comprising the polynucleotide of Claim 1.

6. A host cell comprising the vector of Claim 5.

7. A process for producing a polynucleotide comprising: expressing an RNA from the host cell of Claim 6.

8. A method for the treatment of an individual having need to inhibit a ribosomal polynucleotide comprising: administering to the individual a therapeutically effective amount of a compound that binds to or interacts with a polynucleotide of Claim 1.

9. A method for identifying compounds which interact with and inhibit or activate an activity of the polynucleotide claim 1 comprising the steps of:
contacting a composition comprising the polynucleotide with the compound to be screened under conditions to permit interaction between the compound and the polynucleotide to assess the interaction of a compound, such interaction being associated with a second component capable of providing a detectable signal in response to the interaction of the polynucleotide with the compound; and determining whether the compound interacts with and activates or inhibits an activity of the polynucleotide by detecting the presence or absence of a signal generated from the interaction of the compound with the polynucleotide.

10. An antagonist that inhibits or an agonist that activates an activity a bacterial polynucleotide selected from the group consisting of: a polynucleotide comprising a nucleotide sequence which is at least 70% identical to the nucleotide sequence of SEQ ID NO:1, 2 OR 3, and a polynucleotide comprising a nucleotide sequence as set forth in SEQ ID NO:1, 2 OR 3, by:

binding a compound to a bacterial 50S ribosomal subunit;

binding a compound to a bacterial 70S ribosome

binding a compound to a ribosome under tRNA binding conditions;

binding a compound to a ribosome under tRNA binding conditions using activated ribosomes programmed with messenger RNA such as polyuridylic acid;

binding a compound to *Escherichia coli* 23S rRNA sequence;

binding a compound to *Escherichia coli* 23S rRNA at nucleotides 1971-2607

alteration of RNA secondary structure formed by nucleotides 1971-2607 of *Escherichia coli* 23S rRNA;

alteration of RNA secondary structure formed by domain V of *Escherichia coli* 23S rRNA;

modulation of the binding of SB-328636 (structure 2) to a ribosome;

modulation of the binding of SB-352408 (structure 3) to a ribosome;

modulation of the binding of SB-328636 (structure 2) to a ribosomal 23S RNA;

modulation of the binding of SB-352408 (structure 3) to a ribosomal 23S RNA;

modulation of the binding of SB-328636 (structure 2) to domain V of *Escherichia coli* ribosomal 23S RNA;

modulation of the binding of SB-352408 (structure 3) to domain V of *Escherichia coli* ribosomal 23S RNA;

binding a compound to a ribosomal RNA and a ribosomal protein;

binding a compound to ribosomal protein L4, L32, L33, L2 or L13;

modulating binding of ribosomal protein L4, L32, L33, L2 or L13 to a ribosome;

modulating binding of ribosomal protein L4, L32, L33, L2 or L13 to a ribosomal RNA;

modulation of the binding of a compound to G2061, A2062, or G2502;

modulation of the binding of a pleuromutilin to G2061, A2062, or G2502;

modulation of the binding of a chloramphenicol to G2061, A2062, or G2502;

modulation of the binding of *p*-azidopuromycin G2502;

modulation of the binding of a compound to A2407 and U2408;

modulation of the binding of a pleuromutilin to A2407 and U2408; or

binding a compound to nucleotides of 23S rRNA.

11. A method for the treatment of an individual suspected of being infected by a bacteria using the antagonist or agonist of claim 10.

12. The method of claim 10 wherein said bacteria is selected from the group consisting of a member of the genus *Staphylococcus, Staphylococcus aureus,* a member of the genus *Streptococcus,* and *Streptococcus pneumoniae.*

13. A method for inhibiting an activity of a bacterial ribosome by:
binding a compound to a bacterial 50S ribosomal subunit;
binding a compound to a ribosome under A-site tRNA binding conditions;
binding a compound to a ribosome under A-site tRNA binding conditions using activated ribosomes programmed with polyuridylic acid;
binding a compound to *Escherichia coli* 23S rRNA sequence;
binding a compound to *Escherichia coli* 23S rRNA at nucleotides 1971-2607;
alteration of the RNA secondary structure formed by nucleotides 1971-2607 of *Escherichia coli* 23S rRNA;
alteration of RNA secondary structure formed by domain V of *Escherichia coli* 23S rRNA;
modulation of the binding of SB-328636 (structure 2) to a ribosome;
modulation of the binding of SB-352408 (structure 3) to a ribosome;
modulation of the binding of SB-328636 (structure 2) to a ribosomal 23S RNA;
modulation of the binding of SB-352408 (structure 3) to a ribosomal 23S RNA;
modulation of the binding of SB-328636 (structure 2) to domain V of *Escherichia coli* ribosomal 23S RNA;
modulation of the binding of SB-352408 (structure 3) to domain V of *Escherichia coli* ribosomal 23S RNA;
binding a compound to a ribosomal RNA and a ribosomal protein;
binding a compound to ribosomal protein L4, L32, L33, L2 or L13;
modulating binding of ribosomal protein L4, L32, L33, L2 or L13 to a ribosome;
modulating binding of ribosomal protein L4, L32, L33, L2 or L13 to a ribosomal RNA;
modulation of the binding of a compound to G2061, A2062, or G2502;
modulation of the binding of a pleuromutilin to G2061, A2062, or G2502;
modulation of the binding of a chloramphenicol to G2061, A2062, or G2502;
modulation of the binding of *p*-azidopuromycin G2502;
modulation of the binding of a compound to A2407 and U2408;

modulation of the binding of a pleuromutilin to A2407 and U2408; or
binding a compound to nucleotides of 23S rRNA.

14. The method of claim 13 wherein said bacteria is selected from the group consisting of: a member of the genus *Staphylococcus, Staphylococcus aureus,* a member of the genus *Streptococcus,* and *Streptococcus pneumoniae.*

15. A method for treating an individual infected by a bacteria comprising the steps of contacting an individual suspected to be infected by a bacteria with an antibacterially active amount of a composition comprising a pleuromutilin compound wherein said contacting leads to inhibition of an activity of a bacterial ribosome by:
binding a compound to a bacterial 50S ribosomal subunit;
binding a compound to a ribosome under A-site tRNA binding conditions;
binding a compound to a ribosome under A-site tRNA binding conditions using activated ribosomes programmed with polyuridylic acid;
binding a compound to *Escherichia coli* 23S rRNA sequence;
binding a compound to *Escherichia coli* 23S rRNA at nucleotides 1971-2607
alteration of the RNA secondary structure formed by nucleotides 1971-2607 of *Escherichia coli* 23S rRNA;
alteration of RNA secondary structure formed by domain V of *Escherichia coli* 23S rRNA;
modulation of the binding of SB-328636 (structure 2) to a ribosome;modulation of the binding of SB-352408 (structure 3) to a ribosome;
modulation of the binding of SB-328636 (structure 2) to a ribosomal 23S RNA;
modulation of the binding of SB-352408 (structure 3) to a ribosomal 23S RNA;
modulation of the binding of SB-328636 (structure 2) to domain V of *Escherichia coli* ribosomal 23S RNA;
modulation of the binding of SB-352408 (structure 3) to domain V of *Escherichia coli* ribosomal 23S RNA;
binding a compound to a ribosomal RNA and a ribosomal protein;
binding a compound to ribosomal protein L4, L32, L33, L2 or L13;
modulating binding of ribosomal protein L4, L32, L33, L2 or L13 to a ribosome;
modulating binding of ribosomal protein L4, L32, L33, L2 or L13 to a ribosomal RNA;
modulation of the binding of a compound to G2061, A2062, or G2502;
modulation of the binding of a pleuromutilin to G2061, A2062, or G2502;

modulation of the binding of a chloramphenicol to G2061, A2062, or G2502;

modulation of the binding of *p*-azidopuromycin G2502;

modulation of the binding of a compound to A2407 and U2408;

modulation of the binding of a pleuromutilin to A2407 and U2408; or

binding a compound to nucleotides of 23S rRNA.